British Journal of Venereal Diseases, 1979, **55**, 94–98

Theme 2: Recent advances in the immunology of syphilis

Role of humoral versus cellular mechanisms of resistance in the pathogenesis of syphilis

M. METZGER

From the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland

SUMMARY From experimental work to determine the role of humoral and cell-mediated responses in the pathogenesis of syphilis evidence has been collected to indicate that both humoral and cell-mediated immunity have a protective function in syphilis.

Introduction

It is well known that both rabbits and man infected with *Treponema pallidum* develop resistance to reinfection during the course of the disease (Turner and Hollander, 1957). It has also been shown that a marked resistance to challenge inoculation can be achieved in rabbits by immunisation with the virulent Nichols strain of *T. pallidum* rendered non-infectious either by storage (Metzger *et al.*, 1969; Metzger and Smogór, 1969) or by gamma irradiation (Miller, 1973). The mechanisms underlying immunity in syphilis, however, are poorly understood. Multiple antibodies circulate in the disease, and recent evidence shows that a cell-mediated response develops during the infection. To what extent these two immunological responses protect against syphilis is not clear. The aim of this paper is to give a concise review of the research efforts aimed at clarifying this point.

Humoral immunity

Studies aimed at determining the role of antibodies, or more strictly speaking the role of humoral factors, began in the 1930s and have continued until recently. Experiments of this kind can be divided into three groups: (1) those designed to detect treponemicidal activity in syphilitic serum; (2) those aimed at finding a correlation between the occurrence and level of antibodies and the state of resistance; and (3) those attempting a passive transfer of immunity by immune serum.

Address for reprints: M. Metzger, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, 53–114 Wrocław, Poland

Received for publication 5 July 1978

TREPONEMICIDAL ACTIVITY IN SYPHLITIC SERUM

In his pioneering work Turner (1939) showed that treponemes which had been incubated with sera from untreated syphilitic rabbits lost their virulence and were unable to produce syphilitic lesions. Normal rabbits which had been challenged intracutaneously with such treated treponemes usually failed to develop lesions, or else the resulting lesions had a longer incubation period and remained smaller than those produced by treponemes incubated with normal serum. Later, Turner *et al.* (1948) used serum from syphilitic and non-syphilitic human beings, which gave similar results. Sera from patients with secondary, latent, or tertiary syphilis exerted an inhibitory effect on the development of syphilitic lesions, whereas sera from non-syphilitic persons did not.

These investigations led to the development of the *T. pallidum* immobilisation (TPI) test. In recent years, the treponemicidal activity of syphilitic serum has been studied extensively by Bishop and Miller (1976b) in Los Angeles. Factors have been demonstrated in immune serum which inactivate *T. pallidum* and render it incapable of producing symptomatic infection in rabbits; the appearance, level, and persistence of the factors were found to correlate closely with the development of resistance to symptomatic reinfection after initial *T. pallidum* infection of the source rabbits.

HUMORAL ANTIBODIES AND RESISTANCE AGAINST SYPHILIS

In other studies a correlation was sought between the appearance and level of antibodies reacting in various serological tests and resistance to syphilitic

infection. Experiments of this kind were performed in several laboratories and all gave negative results (Izzat *et al.*, 1971; Magnuson *et al.*, 1951; McLeod and Magnuson, 1953; Metzger *et al.*, 1969; Metzger and Smogór, 1969; Miller *et al.*, 1953). None of the investigators could show a correlation between the degree of resistance and the titre of antibodies, even in the case of antibodies clearly associated with resistance, such as immobilisins. Our own findings also show this lack of correlation (Table 1).

A total of 302 rabbits were immunised with a treponemal vaccine developed in our laboratory (Metzger and Smogór, 1969). On the basis of the results of the infectivity test the rabbits were classified as completely immune, partially immune, and non-immune. Assay of circulating antibodies was conducted repeatedly during immunisation and after challenge by a variety of serological tests for syphilis. Only those results, however, which were obtained five weeks after completion of the immunisation (that is, just before challenge inoculation) are recorded in Table 1. Both the mean values and ranges of serum titres found in the various serological tests were nearly the same in the three groups of rabbits, which showed different degrees of susceptibility to infection. It was particularly interesting to note that none of the immunised rabbits responded with the production of TPI antibody—not even those which proved completely resistant to challenge. Close inspection of the pertinent protocols has also shown no relation between the serological response of individual animals and their immune status; rabbits whose sera exhibited the same or nearly the same degree of serological reactivity behaved differently on challenge, being either completely free of syphilitic infection or showing asymptomatic or even symptomatic infection.

PASSIVE TRANSFER OF RESISTANCE BY IMMUNE SERA

In recent years the most extensive studies of this type were carried out in rabbits by Bishop and Miller (1976a), Perine *et al.* (1973), Sepetjian *et al.* (1973), and Weiser *et al.* (1976). Their results were similar; injections of hyperimmune serum delayed the appearance and diminished the severity and duration of syphilitic lesions which developed after challenge with *T. pallidum*. Thus, these studies have suggested that a humoral mechanism of resistance may have been transferred by passive immunisation, despite vast amounts of serum (ranging up to 400 ml per rabbit) and prolonged daily administration (over 37 days) having been needed to bring about this incomplete protective effect.

SUMMARY OF ROLE OF HUMORAL FACTORS

Syphilitic serum exerts a lethal effect on treponemes and immunity to syphilis can be passively transferred by immune serum; no true correlation, however, has been established between the appearance and level of a variety of syphilitic antibodies—even those clearly associated with resistance—and the degree of resistance. Despite this limitation these findings have suggested that humoral immunity is operative in syphilis. No evidence has been shown, however, that antibodies are concerned in the immune reaction and no protective antibody has been demonstrated.

Cell mediated immunity

Preliminary experiments have been carried out to discover whether and how the cell-mediated response to treponemal antigens develops in rabbits during syphilitic infection and following artificial immunisation (Metzger *et al.*, 1977). The macrophage migration inhibition (MMI) test was used to demonstrate this type of immunological response.

Lymphocytes of syphilitic rabbits exhibited a well marked capacity to inhibit macrophage migration as early as one month after onset of the infection (Table 2). The level of this response rose slowly during the next two months, reaching an average value of about 50% inhibition in the third month. Between the fourth and sixth months,

Table 1 *Results of studies to determine correlation between syphilis immunity and humoral antibody response*

*Immune status of rabbits**	*Reciprocal serum titres in serological tests*											*Opsonophagocytic test (% positive sera)*
	VDRL		*TPI*	*FTA*		*Agglutination*		*Antiglobulin agglutination*		*TPHA*		
	Mean	*Range*		*Mean*	*Range*	*Mean*	*Range*	*Mean*	*Range*	*Mean*	*Range*	
Immune	2	0–32	0	54	0–320	186	0–1280	80	0–160	170	0–2560	52
Partly immune	3	0–64	0	57	0–320	180	0–1280	64	0–80	200	0–2560	31
Non-immune	2	0–32	0	43	0–320	110	0–640	108	0–320	170	0–1280	42

*Immune means no lesions and lymph node transfers negative; partly immune, no lesions but lymph node transfers positive; and non-immune, lesions at one or more challenge sites

Table 2 *MMI response in rabbits experimentally infected with* Treponema pallidum

Months after infection	*No. of rabbits tested*	*Mean % inhibition*	*SD*
1/2	16	4·7	10·5
1	18	42·9	19·7
2	18	37·2	21·6
3	14	46·3	18·4
4	19	19·8	20·7
6	11	4·8	9·5
8	16	45·8	19·2
11	10	49·7	15·2
12	10	48·3	13·9
13	11	54·2	16·5
15	10	68·2	11·8
16	9	72·0	15·5
26	6	74·3	9·2

however, a distinct weakening of this capacity occurred, followed by a slow but steady increase during the next two years, that is, until the end of the observation period.

The development of the MMI response in rabbits artificially immunised with non-viable treponemes is shown in Table 3. As early as four weeks after the start of immunisation, the lymphocytes of the rabbits exerted a marked inhibitory effect on the migration of macrophages. This capacity reached a peak value (about 40% inhibition) by the second month, remained at this level with slight fluctuations during the subsequent six months and thereafter showed a slow decrease.

These results clearly show that during syphilitic infection and following artificial immunisation the cell-mediated response to treponemal antigens develops in the host. The demonstration of this type of immunological response does not, however, directly indicate that this reaction plays a protective role in syphilis. Thus, to show this, experiments were designed following essentially the same research plan as that used for demonstrating the role of humoral factors.

Table 3 *MMI response in rabbits immunised with non-viable* Treponema pallidum

Months after start of immunisation	*No. of rabbits tested*	*Mean % inhibition*	*SD*
1	16	29·2	16·4
2	17	39·6	18·2
3	14	35·5	14·6
4	5	37·4	16·2
6	5	35·4	12·7
7	5	34·8	13·4
8	5	33·2	12·5
10	4	19·2	8·4
11	6	16·3	6·8

TOXIC EFFECT OF IMMUNE LYMPHOCYTES

The results of these studies showed that extracts of lymphocytes from immune rabbits exerted an *in-vitro* toxic effect on *T. pallidum* whereas those from non-immune animals did not (unpublished data).

MACROPHAGE MIGRATION AND RESISTANCE TO INFECTION

Attempts to correlate the appearance and level of the cell-mediated immunological response with resistance to infection gave rather equivocal results (Metzger *et al.*, 1977) (Table 4). After being immunised, a group of 11 rabbits was infected intradermally with *T. pallidum*. The MMI test was performed once during the immunisation course and twice after completion. The results of the test, performed just before challenge, were compared with the immune status of the animals, as shown by the infectivity test. The post-immunisation level of the MMI response varied greatly in individual animals, the percentage inhibition ranging from 0% to 73% (Table 4). When the results of the MMI test are compared with those of the infectivity test, however, no strict correlation was shown between the level of the MMI response of the individual animals and their ability to combat the infection. Rabbits whose lymphocytes inhibited migration of macrophages to a varying degree, and even the rabbit (no. 2100) whose lymphocytes did not exhibit this ability on repeated testing, proved immune or partly immune after challenge. As a group, however, the lymphocytes from immune and partly immune rabbits exerted a much greater

Table 4 *Results of attempt to correlate levels of MMI response with resistance to infection with* Treponema pallidum

	% inhibition (in weeks after start of immunisation)			
Rabbit no.	4	8	11	*Immune status**
2118	16	75	73	
2121	12	44	56	
2152	12	53	47	immune
2143	0	38	45	
2122	0	45	38	
2100	6	0	0	
724	32	54	46	
2101	45	50	42	partly immune
2040	53	54	29	
2054	37	31	10	non-immune
2133	0	0	0	

*Immune means no lesions and lymph node transfers negative; partly immune, no lesions but lymph node transfer positive; non-immune, lesions at one or more challenge sites

inhibitory effect on the migration of macrophages compared with those from rabbits of the non-immune group.

EFFECT OF IMMUNOSUPPRESSION

The clinical course of syphilis has been compared in rabbits treated with immunosuppressive agents (Pacha *et al.*, 1978) and in those not so treated. These experiments were designed on the assumption that a milder course of the disease in the rabbits with experimentally induced immunosuppression would indicate a pathogenic role of humoral or cell-mediated immunity or both. On the other hand, a more severe course of the disease under these experimental conditions could be interpreted as evidence of the protective role of humoral or cell-mediated immunity or both. The results of this study suggested the latter possibility.

Treatment of syphilitic rabbits with immunosuppressive agents (cyclophosphamide or 5-fluorouracil) resulted in almost complete abolition of the cell-mediated response (as measured by the MMI test), while the humoral response (as measured by the Venereal Disease Research Laboratory (VDRL), fluorescent treponemal antibody (FTA), and *T. pallidum* haemagglutination (TPHA) tests), was not affected by this procedure (Table 5). In the group of rabbits given immunosuppressors syphilitic lesions appeared not only at the challenge sites as usual but, after healing of the primary lesions, multiple multiform secondary lesions developed over the entire dorsal area. These results may be interpreted as demonstrating the protective role of the cell-mediated response in syphilis.

TRANSFER OF RESISTANCE BY IMMUNE LYMPHOCYTES

The possibility of such a protective role has been reinforced by experiments in which immunity was successfully transferred from immune donor rabbits to normal recipient rabbits with lymphocytes extracted from the lymph nodes of immune donors (Metzger and Smogór, 1975). Lymphocytes from those donors which had had previous experience with *T. pallidum*, (that is, those immunised with non-viable *T. pallidum* or cured of syphilitic infection by penicillin treatment) conferred a state of resistance to challenge on most of the recipient rabbits (Table 6); this was demonstrated either by the absence of lesions at the challenge sites or by the reduced number and delayed incubation period of lesions compared with those in the controls. Some of the donor and recipient rabbits were also tested for humoral and cell-mediated response by the TPI and MMI tests respectively. The lymphocytes of the donors were active in inhibiting migration of macrophages, and this ability was found in the recipients after lymphocyte transfer (Table 6). In contrast, however, although the rabbits of the immunised group responded with TPI antibody this response could not be transferred by lymphocytes into recipient rabbits.

Although these experiments could not be performed under conditions which guaranteed the survival of the transferred lymphocytes in recipient rabbits (syngeneic rabbits were not available), evidence of partial protection was achieved in most of the passively immunised animals. Furthermore, every step of the procedure used in this study was

Table 5 *Clinical course of syphilis in immunosuppressed rabbits*

Syphilitic rabbits	*Humoral response (VDRL, TPI, FTA, TPHA tests)*	*Cell-mediated response (MMI test)*	*Clinical course*
Untreated	+	+	Typical syphilitic lesions at challenge sites
Treated with immunosuppressors (cyclophosphamide or 5-fluorouracil)	+	0	Exacerbated (multiple disseminated secondary syphilitic lesions)

+Positive

Table 6 *Transfer of syphilitic immunity by immune lymph node lymphocytes*

Donor rabbits				*Recipient rabbits*			
Pretreatment	*No. of rabbits*	*Humoral response (TPI test)*	*Cell-mediated response (MMI test)*	*No. of rabbits*	*Humoral response (TPI test)*	*Cell-mediated response (MMI test)*	*No. of immune rabbits*
Immunised with non-viable *T. pallidum* or infected with *T. pallidum* and cured	17	+	+	19	0	+	16
None	9	0	0	9	0	0	0

+Positive

arbitrarily established; different experimental conditions may give more complete protection and this is now being studied in our laboratory. Despite these limitations, the findings of this study together with those previously described strongly indicate the protective role of cell-mediated immunity in syphilis. Baughn *et al.* (1977), however, reported the inability of spleen cells from immune rabbits to confer immunity to challenge with *T. pallidum*. I think that this failure will be clarified by further experiments on a more extended basis.

Conclusion

Both humoral and cell-mediated responses are concerned in the pathogenic mechanisms in syphilis. Humoral and cell-mediated immunity, however, can be separated only for purposes of analysis, since in the organism they act in concert to protect the host against infection. The interplay between humoral and cellular immunity is important in producing the great variability of clinical symptoms in syphilis and may even contribute to the recurrence of the disease.

References

Baughn, R. E., Musher, D. M., and Simmons, C. B. (1977). Inability of spleen cells from chancre-immune rabbits to confer immunity to challenge with *Treponema pallidum*. *Infection and Immunity*, **17**, 535–540.

Bishop, N. H., and Miller, J. N. (1976a). Humoral immunity in experimental syphilis. I. The demonstration of resistance conferred by passive immunization. *Journal of Immunology*, **117**, 191–196.

Bishop, N. H., and Miller, J. N. (1976b). Humoral immunity in experimental syphilis. II. The relationship of neutralizing factors in immune serum to acquired resistance. *Journal of Immunology*, **117**, 197–207.

Izzat, N. N., Knox, J. M., Dacres, W. G., and Smith, E. B. (1971). Resistance and serological changes in rabbits immunized with virulent *Treponema pallidum* sonicate. *Acta dermatovenerological (Stockholm)*, **51**, 157–160.

Magnuson, H. J., Thompson, F. A., and McLeod, C. P. (1951). Relationship between treponemal immobilizing antibodies and acquired immunity in experimental syphilis. *Journal of Immunology*, **67**, 41–48.

McLeod, C. P., and Magnuson, H. J. (1953). Production of immobilizing antibodies unaccompanied by active immunity to *Treponema pallidum* as shown by injecting rabbits and mice with the killed organisms. *American Journal of Syphilis, Gonorrhoea, and Venereal Diseases*, **37**, 9–22.

Metzger, M., Michalska, E., Podwińska, J., and Smogór, W. (1969). Immunogenic properties of the protein component of *Treponema pallidum*. *British Journal of Venereal Diseases*, **45**, 299–304.

Metzger, M., Podwińska, J., and Smogór, W. (1977). Cell-mediated immunity in experimental syphilis in rabbits. *Archivum Immunologiae et Therapiae Experimentalis*, **25**, 25–34.

Metzger, M., and Smogór, W. (1969). Artificial immunization of rabbits against syphilis. I. Effect of increasing doses of treponemes given by the intramuscular route. *British Journal of Venereal Diseases*, **45**, 308–312.

Metzger, M., and Smogór, W. (1975). Passive transfer of immunity to experimental syphilis in rabbits by immune lymphocytes. *Archivum Immunologiae et Therapiae Experimentalis*, **23**, 625–630.

Miller, J. N. (1973). Immunity in experimental syphilis. VI. Successful vaccination of rabbits with *Treponema pallidum*, Nichols strain, attenuated by gamma irradiation. *Journal of Immunology*, **110**, 1206–1215.

Miller, J. N., Fazzan, F. P., and Whang, S. J. (1963). Studies on immunity in experimental syphilis. II. *Treponema pallidum* immobilization (TPI) antibody and the immune response. *British Journal of Venereal Diseases*, **39**, 199–203.

Pacha, J., Metzger, M., Smogór, W., Michalska, E., Podwińska, J., and Ruczkowska, J. (1979). Effect of immunosuppressive agents upon course of experimental syphilis in rabbits. *Archivum Immunologiae et Therapiae Experimentalis*, **27**, 45–51.

Perine, P. L., Weiser, R. S., and Klebanoff, S. J. (1973). Immunity to syphilis. I. Passive transfer in rabbits with hyperimmune serum. *Infection and Immunity*, **8**, 787–790.

Sepetjian, M., Salussola, D., and Thivolet, J. (1973). Attempt to protect rabbits against experimental syphilis by passive immunization. *British Journal of Venereal Diseases*, **49**, 335–337.

Turner, T. B. (1939). Protective antibodies in the serum of syphilitic rabbits. *Journal of Experimental Medicine*, **69**, 867–871.

Turner, T. B., Hardy, P. H., jun., Newman, B., and Nell, E. E. (1973). Effects of passive immunization on experimental syphilis in the rabbit. *Johns Hopkins Medical Journal*, **133**, 241–251.

Turner, T. B., and Hollander, D. H. (1957). Biology of the treponematoses, *Monograph Series No.* 35, p.123. WHO: Geneva.

Turner, T. B., Kluth, F. C., McLeod, C., and Winsor, C. P. (1948). Protective antibodies in the serum of syphilitic patients. *American Journal of Hygiene*, **48**, 173–176.

Weiser, R. S., Erickson, D., Perine, P. L., and Pearsall, N. N. (1976). Immunity to syphilis: Passive transfer in rabbits using serial doses of immune serum. *Infection and Immunity*, **13**, 1402–1407.